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(54) Title: GLUTHIONE ANALOGUES AND THEIR USE AS ANTIOXIDANTS

(57) Abstract: Glutathione analogues are disclosed. These have Formula (I): X-Glu-Y-Gly-Z, wherein Glu is glutamyl or γ-glutamyl, and Gly is glycine or glycyl, X is selected from the group consisting of H-; (O-R)tyrosyl; biotinyl-(O-R)tyrosyl; anthraniloyl-(O-R)tyrosyl; (O-R) tyrosyl-aminohexanoyl-; and 5-alkoxy tryptophyl-; Y is selected from the group consisting of cysteinyl, serinyl, methionyl or selenocysteinyl; and Z is selected from the group consisting of -H; (O-R)tyrosine; and aminohexanoyl-(O-R)tyrosine, and wherein each amino acids can be either a L- or a D- enantiomer; the peptide bonds may optionally be methylated, and the carboxyl groups are free acids, amides, or mono- or di-alkyl esters, and R is selected from alkyl, alkenyl, aryl and aryl containing one or several heteroatoms in the ring(s); with the provisio that X is not H when Y is L-cysteinyl and Gly is L-glycyl. Glutathione analogues for use as antioxidants, e.g. in cosmetics and pharmaceutical compositions, use of the gluthathione analogues for the manufacture of a medicament for the prophylactic and/or therapeutic treatment of a disease or disorder associated with oxidative degeneration of cells, such as Alzheimer's disease or Parkinson's disease, and a method of such treatment, are also described.

GLUTHIONE ANALOGUES AND THEIR USE AS ANTIOXIDANTS

The present invention relates to a new group of glutathione analogues and pharmaceutical compositions containing them as well as to the glutathione analogues for use as antioxidants. The invention also relates to the use of the glutathione analogues for the manufacture of a medicament for the prophylactic and/or therapeutic treatment of a disease or disorder associated with oxidative degeneration of cells and to a method of such treatment.

Background

Glutathione is widely distributed in human body, mainly in red blood cells, liver, brain etc., and its concentration in most mammalian cells is 1 to 10 mM (Meister,1989; Anderson, 1997). Glutathione in its reduced form, GSH, is a natural tripeptide with the sequence L-γ-glutamyl-L-cysteinyl-glycine, and thus it contains a sulphydryl group (Kosower,1976). The sulphydryl group enables the transition between GSH and its disulfide dimer (oxidized glutathione, GSSG). Glutathione exists primarily as GSH and the steady state within e.g. red blood cells commonly maintains a ratio of about 100: 1 of GSH/GSSG.

The isopeptidic nature of the γ-glutamyl linkage renders GSH resistant to cleavage by most peptidases. The electronic structure of the sulfur atom accounts for high reactivity of the thiol group towards nucleophilic addition, redox reactions (e.g. via radical mechanism) and metal chelation. All these properties underlay the detoxifying and antioxidant effects of GSH and help GSH to fulfill important functions in the cells. Known functions are as follows (Meister, 1989; Anderson, 1997; Griffith, 1999; Briviba, 1999; Voehringer, 1999).

a) in the red blood cells, in which GSH is present in high concentration, it prevents the denaturation of hemoglobin and reduces methemoglobin back to hemoglobin,
b) GSH works also as a scavenger of free radicals and peroxides via co-operation with a selenium-containing enzyme (glutathione peroxidase),
c) GSH is the major non-enzymatic cellular antioxidant and plays a major role in cellular defenses against oxidative and nitrosative stress,
d) GSH is necessary in synthesis of leukotrines and prostaglandins, and in detoxification of several xenobiotics by glutathione S-transferases,
e) GSH is involved in transport of nitric oxide and in regulation of several enzymes (glutathionylation of enzymes),
f) GSH works also as molecular regulator of cellular physiology (it plays important role in BCL-2's ability to suppress apoptosis).

The above presented crucial spectrum of GSH functionality is the main reason why there has been an increasing interest in GSH as the most abundant non-protein thiol in mammalian cells. Perhaps one of its most important functions is the protection against oxidative damage caused by reactive oxygen species (ROS) that are generated both in normal metabolism and diseases. GSH reacts rapidly and non-enzymatically with hydroxyl radical, and with cytotoxic products, peroxynitrite and N_2O_3 (Kalyanaraman, 1996; Luperchio, 1996; Briviba, 1999). Thus, due to the crucial role of GSH in detoxification of electrophiles, oxidative and nitrosative stress factors, strategies for pharmacologically maintaining or increasing tissue levels of GSH have received high attention. However, the administration of GSH itself is not a very effective way to increase cellular level of GSH for many reasons, including poor transport into cells and extracellular degradation (Meister, 1991; Anderson, 1997). It is also known, that glutathione analogues are characterized by potential pharmacological properties (Meister,1995; Lucente,1998).

It would be of great medical interest to find some analogues of GSH which have substantially higher penetration ability and antioxidative potency compared to GSH.

Description of the invention

The present invention provides GSH-like new compounds with substantially higher hydrophobicity and antioxidativity compared with natural GSH. The compounds of the invention are glutathione analogues of the formula

X-Glu -Y-Gly -Z

wherein Glu is glutamyl or γ-glutamyl, and Gly is glycine or glycyl,

X is selected from the group consisting of H-; (O-R)tyrosyl; biotinyl-(O-R)tyrosyl; anthraniloyl-(O-R)tyrosyl; (O-R) tyrosyl-aminohexanoyl-; and 5-alkoxy tryptophyl-;

Y is selected from the group consisting of cysteinyl, serinyl, methionyl or selenocysteinyl; and

Z is selected from the group consisting of -H; (O-R)tyrosine; and aminohexanoyl-(O-R)tyrosine,

and wherein each amino acids can be either a L- or a D- enantiomer; the peptide bonds may optionally be methylated, and the carboxyl groups are free acids, amides, or mono- or di-alkyl esters, and R is selected from alkyl, alkenyl, aryl and aryl containing one or several heteroatoms in the ring(s)

with the provisio that X is not H when Y is L-cysteinyl and Gly is L-glycyl.

This provisio is made to exclude natural glutathione.

Preferred glutathione analogues of the invention are those wherein each alkyl group and the alkyl of the alkoxy group is selected from alkyl groups with from 1 to 20 carbon atoms; each

alkenyl group is selected from alkenyl groups with 2 to 20 carbon atoms; each aryl group is selected from phenyl, benzyl, tolyl, xylenyl, naphtyl, anthranoyl or their substituted analogues, and each aryl group containing one or several heteroatoms in the ring(s) is selected from pyrrol, imidazole, thiazole, pyridine, pyrimidine, quinoline, indole, purine and their substituted analogues.

At present, the most preferred gutathione analogues of the invention are those wherein each alkyl group is methyl and the alkoxy group is methoxy.

Examples of specific compounds of the invention are Tyr(Me)-γ-Glu-Cys-Gly, γ-Glu-Cys-Gly-Tyr(Me), D-Tyr(Me)-γ-Glu-Cys-Gly amide, and γ-Glu-Cys-Gly-Tyr(Me) amide.

Other specific compounds of the invention are selected from biotinyl and anthraniloyl N-terminally substituted Tyr(Me)-γ-Glu-Cys-Gly ; γ-Glu-Cys-Gly-Tyr(Me); D-Tyr(Me)-γ-Glu-Cys-Gly amide; and γ-Glu-Cys-Gly-Tyr(Me) amide.

In addition to the glutathione analogues of the invention, the invention comprises other aspects based on the properties of the compounds.

Thus, one aspect of the invention is directed to a glutathione analogue of the invention for use as an antioxidant. The glutathione analogues of the invention are readily soluble in water and are as purified white powders. Therefore they will be useful in cosmetics and skin cream or lotion for e.g. UV-protection. Today, there are a number products in the cosmetic and topical medical products where different carotenoids are used as antioxidants. However, the use of carotenoids in such products is limited by their red to yellow colors. The antioxidants of the invention may find use also in feed, foodstuff and oils. An important utility of the antioxidant compounds of the invention is their use *in vivo*.

Therefore, another aspect of the invention is directed to the use of a glutathione analogue according to the invention for the manufacture of a medicament for the prophylactic and/or therapeutic treatment of a disease or disorder associated with oxidative degeneration of cells. For example, the disease or disorder is selected from Alzheimer's disease, Parkinson's disease, sunburn, adult respiratory distress syndrome (ARDS), cystic fibrosis, idiopathic pulmonary fibrosis (IPF), asthma, hyperoxia, HIV infection, influenza, Sjögren's syndrome, heart disease, epilepsy (Unverricht-Lundborg type), chronic bronchitis, and cancer.

Yet another aspect of the invention is directed to a method of treating a disease or disorder associated with oxidative degeneration of cells in an animal or human comprising administration of a prophylactically or therapeutically effective amount of a glutathione analogue according to the invention to said animal or human. The prophylactically or therapeutically effective amount of a glutathione analogue according to the invention will be determined by the manufacturer of a pharmaceutical composition, based on dose-response experiments and prior experience. The

method of the invention will be used when animals or humans have inborn errors of GSH metabolism, or to counteract or stop oxidative degeneration of cells, e.g. when animals or humans suffer from or are likely to get a disease or disorder selected from the group consisting of Alzheimer's disease, Parkinson's disease, sunburn, adult respiratory distress syndrome (ARDS), cystic fibrosis, idiopathic pulmonary fibrosis (IPF), asthma, hyperoxia, HIV infection, influenza, Sjögren's syndrome, heart disease, epilepsy (Unverricht-Lundborg type), chronic bronchitis, and cancer.

Still another aspect of the invention is directed to a pharmaceutical preparation comprising a glutathione analogue according to the invention, and a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier is selected with regard to the intended rout of administration from carriers known in the art, such as aqueous solutions e.g. isotonic saline solution for infusion, gelatin and liposomes for incapsulation of the active ingredient, excipients for tablets and lozenges. Further, the pharmaceutically acceptable carriers in pharmaceutical preparation of the present invention may be selected from cell-penetrating peptides, such as transportan, penetratin, Tat peptide (Lindgren, 2000), which will be used to enhance cellular uptake of glutathione analogues. Cell-penetrating peptides will be attached to the glutathione analogues through disulphide bond, which will be cleaved in the intracellular milieu.

The present invention will now be further illustrated by reference to the following description of drawings, experiments and specific embodiments of the invention, which are not to be considered as limitations to the scope of the invention defined by the claims.

Short description of the drawings

Figure. 1. Comparison of antioxidative potency of melatonin (filled square), UPF1 (Tyr(Me)-γ-Glu-Cys-Gly) (filled triangular) and glutathione (empty square).

Figure 2. Effect of antioxidants glutathione (GSH, 1.5 mM) and UPF1 (0.025 mM) on the stimulated by 10^{-7}M of Aβ(25-35) adenylate cyclase activity in membranes of rat frontal cortex. (0% corresponds to the basal value of adenylate cyclase activity in the absence of ligands).

Figure 3. Lipid peroxidation in striatum after the treatment with MPTP, or with pretreatment with melatonin or UPF1.

Figure 4. Lipid peroxidation in hippocampus after the treatment with MPTP, or with pretreatment with melatonin or UPF1.

Figure 5. Glutathione redox ratio in striatum after the treatment with MPTP, or with pretreatment with melatonin or UPF1.

Figure 6. Total antioxidative activity in striatum after the treatment with MPTP, or with pretreatment with melatonin or UPF1.

Figure 7. Glutathione redox ratio in frontal cortex after the treatment with MPTP, or with pretreatment with melatonin or UPF1.

Description of experiments

The following peptides were synthesized and used in the experiments:

UPF1: O-methyl-L-tyrosinyl-γ-L-glutamyl-L-cysteinyl-glycine

(Tyr(Me)-γ-Glu-Cys-Gly)

UPF2: γ-L-glutamyl-L-cysteinyl-glycine-(O-methyl)-L-tyrosine

(γ-Glu-Cys-Gly-Tyr(Me))

UPF3: O-methyl-D-tyrosinyl-γ-L-glutamyl-L-cysteinyl-glycine amide

(D-Tyr(Me)-γ-Glu-Cys-Gly amide)

UPF4: γ-L-glutamyl-L-cysteinyl-glycine-(O-methyl)-L-tyrosine amide

(γ-Glu-Cys-Gly-Tyr(Me) amide)

Synthesis of peptides

UPF1 and its analogues were synthesized in a stepwise manner in a 0.1 mmol scale on an Applied Biosystem Model 431A peptide synthesizer on a solid support using N,N'-dicyclohexylcarbodiimide-hydroxybenzotriazole activation strategy. *tert*-Butyloxycarbonyl amino acids were coupled as hydroxybenzotriazole esters to a phenylacetamidomethyl-resin (0.6 mmol amino groups per gram resin, Novabiochem, Switzerland) to achieve the C-terminal free carboxylic acid or to a *p*-methylbenzylhydrylamine, MBHA, resin (1.1 mmol of amino groups/g, Bachem, Switzerland) to obtain C-terminally amidated peptides. The peptides were finally cleaved from the resin with liquid HF at 0°C for 30 min. Deprotection of the side chains, cleavage of the peptides and purification on HPLC have been described in detail earlier (Langel, 1992). The purity of the peptide was >99% as demonstrated by HPLC on an analytical Nucleosil 120-3 C_{18} reversed-phase column (0.4 cm x 10 cm). The molecular masses of the peptides were determined by a plasma desorption mass spectrometry (Bioion 20, Applied Biosystems) and the calculated values were obtained in each case.

Hydrophobicity of GSH and UPF1

Partition in water/octanol system was determined for GSH and UPF1. The amounts of UPF1 were found higher in octanol (c_2) than in water (c_1). Partition coefficient value ($P=c_1/c_2$) for UPF1, P=3.6 was found less than for GSH, P=12.4, which suggests that UPF1 peptide may enter the cell plasma membrane.

Cellular penetration and transport of UPF1.

Studies of the internalisation of UPF1 into cells require their visualisation e.g. including a label into its sequence. We used biotin or anthranilic acid (Abz) to N-terminally label UPF1 and its analogues.

Internalisation of biotinylated peptides was followed using indirect fluorescence method, which included a treatment of cells with biotinyl-peptides, permeabilisation of cells and subsequent treatment of cells with avidin or streptavidin conjugated with fluorochromes for visualisation. Internalisation of Abz-labeled peptides was followed by direct fluorescence method.

To enhance intracellular delivery of UPF1 and its analogues we synthesised cell penetrating constructs between transportan analogues and UPF1. The cysteine containing transport peptides were linked by a disulphide bond to UPF1. The disulphide bond is quickly reduced in the intracellular milieu, leading to the dissociation of the UPF1 peptide.

Hydroxyl radicals eliminating ability

It has been well documented that oxidative stress via free radicals, ROS, reactive nitrogen species (RNS), underlies the development of Alzheimer's disease, atherosclerosis, coronary heart disease, parkinsonism, cancer etc. For instance, during the last decade it is established that ROS, lipid peroxidation and oxidatively modified low density lipoproteins (oxLDL) have a crucial role in the etiology of atherogenesis and its associated disorders, which include coronary heart disease, stroke, ischaemic dementia etc. Consequently, the design, studies and applying of new antioxidant compounds (neuroprotectants) will have impact in development of novel therapeutic approaches for the treatment of several diseases, including Alzheimer's and Parkinson's disease (Meister, 1991; Lucente, 1998; Behl, 1999).

GSH is known as a major cellular antioxidant. One of the new analogue of GSH (called UPF1) of the invention has been tested to verify, at first, its antioxidant effect (hydroxyl radical scavenging potency), and secondly, to compare its antioxidant potency with antioxidant potency of melatonin and GSH, known as two natural and important scavenger of hydroxyl radicals in the cells.

The procedures were performed by using terephthalic acid as a chemical dosimeter for hydroxyl radicals (Baretto, 1995). The terephthalic acid solution contained 10 mmol terephthalic acid in a sodium phosphate buffer at pH 7.5 and UPF1 or GSH or melatonin, respectively. The hydroxyl radicals were generated via adding $CuSO_4$ (10 micromol) and free radical suppressing influence was measured with a spectrofluorometer (Perkin Elmer LS5) at 312 nm excitation and 426 nm emission at pH 7.5.

Under the described conditions UPF1, an analogue of GSH, possessed a clear hydroxyl radicals scavenging (antioxidative) nature (Figure 2.). Comparison of antioxidative potency of UPF1 with melatonin showed that melatonin (IC_{50} = 9.5 μM) and UPF1 (IC_{50} = 20.5 μM) are very potent hydroxyl radicals scavenging reagents already at very low concentrations. GSH showed significantly lower scavenging efficiency (IC_{50}=1.3 mM).

The results revealed that the new analogue of glutathione according to the invention had about 60 times lower value of IC_{50} compared to glutathione (i.e. its antioxidativity is about 60 times higher), and the efficiency of UPF1 is comparable with that of the very good hydroxyl radical scavenger, melatonin.

Adenylate Cyclase Assay

Membranes of frontal cortex were prepared from Wistar rats (200-300g), according to previously published procedures (Valkna, 1995). Homogenates (in 8 mM HEPES-Na, pH 7.4) of precooled ventral hippocampus were diluted, stirred on ice for 30 min and centrifuged for 6 min at 1600xg. The pellets were resuspended in ice-cold protein-buffer (4 mM HEPES-Na, 1.5 mM theophylline, 8.25 mM $MgCl_2$, 0.75 mM EGTA, 7.5 mM KCl, 100 mM NaCl, pH 7.4) to a final protein concentration of 0.6-0.8 mg/ml. The basal adenylate cyclase activity was assayed at 0.04 mg/ml of membrane protein in reaction-buffer, additionally containing (in protein buffer) 100 μg/ml bacitracin, 0.03 % bovine serum albumin, 10 mM phosphoenol-pyruvate and 30 μg/ml pyruvate kinase (Valkna,1995). In all experiments the peptides, dissolved in the reaction buffer, were added to the assay mixture 2 min before the reaction was initiated by 10 mM ATP/10 μM GTP. The reaction at 30°C was terminated after 15 min by the addition of 100 mM EDTA, followed by boiling samples for 3 min. The cyclic AMP content in the tubes was measured by a competitive protein saturation assay using cyclic AMP-binding protein from bovine adrenal cortex (Broulliet,1990). The basal level of the adenylate cyclase activity in frontal cortex was 76 ± 1 pmol cyclic AMP/min/mg protein. The protein content of the membrane preparations was determined according to Lowry (Lowry,1951).

Incubation of tissue membranes with antioxidants and Aβ(25-35)

We have examined the effects of the antioxidant glutathione and UPF1 peptide on the basal activity of adenylate cyclase as well as antioxidant induced alterations in the modulation of adenylate cyclase activity by Aβ(25-35). The effect was measured as a difference in the amount of cAMP, produced by membranous adenylate cyclase in the presence or absence of 10^{-7}M Aβ(25-35) and in the conditions where glutathione (final concentration 1.5 mM) or UPF1 (0.0025 mM) were added to the medium before the peptide.

Effect of GSH and UPF1 on adenylate cyclase activity

Reduced glutathione, GSH, has previously been shown to protect SK-N-SH human neuroblastoma cells from Aβ(25-35) toxicity (Gridely,1998). We examined the effects of GSH and UPF1 on the stimulation of adenylate cyclase by Aβ(25-35) in rat cerebral cortical membranes. It was shown that Aβ(25-35) at 0.1 μM stimulates the basal activity of adenylate cyclase by 23 ± 7 % in the membranes studied (Soomets,1999).

This activation was completely abolished by pre-treatment of membranes with 1.5 mM of GSH or with 0.025 mM of UPF1 (Figure 2.). The results suggest that stimulation of adenylate cyclase activity by Aβ(25-35) may involve a ROS generation as a mechanism. UPF1 appears to be more potent antioxidative agent against ROS-mediated adenylate cyclase stimulation than GSH.

Experiments on MPTP Parkinson's disease model

A number of observations have been referred to role of oxidative stress in the pathogenesis of Parkinson's disease. These include:

1) decreased nigral glutathione (Perry, 1982; Ferrano,1986; Riederer,1989; Sofic,1992, Jenner,1994);
2) increased lipid peroxidation in the substantia nigra (Dexter, 1989);
3) increased protein modification by 4-hydroxynonenal (Yorikata, 1996).

Thus, deficiency of the antioxidant GSH in brain appears to be associated with several diseases characterized by neurodegenerativity (Cookson,1999; Dringen,1999; Castange,1999; Love,1999; Browne,1999; Gurwitz, 1999; Raina,1999).

The MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model of Parkinson's disease is a prominent model used in literature (Ferrano,1986; Sundström,1987; Acuna-Castroviejo,1997; Grunblatt,1999; Miller,1999). Thus, in the next experiments we studied the neuroprotective effects of UPF1 using the MPTP model of Parkinson's disease. The intracerebroventricular injection of MPTP and antioxidative peptides (melatonin and UPF1) were performed as follows.

Mice were anesthetized with ether and mounted in the stereotactic frame (Stoelting). Upper jaw was fixed with special tool for mice. A longitudinal skin incision (0.5 cm) was performed and the skull was exposed and cleaned from connective tissue. Thereafter one hole was drilled according to the coordinates of Franklin and Paxinos (Paxinos and Franklin "The Mouse Brain in Stereotactic Coordinates"). Coordinates were chosen to be reached with needle to the 3rd ventricle at the midline (from Bregma AP: - 2.06 mm, DV: 2.2 mm, ML: 0). Injection needle was lowered and after waiting for 3 minutes syringe pump was switched on (speed 1 mikroliter/5 min). After completing the procedure we waited for another 5 minutes before removing the needle from the

ventricle. The skin wound was closed with clips and the animals were taken off from the stereotactic frame.

MPTP is a drug which is widely used to reproduce Parkinson's disease symptoms in animals, since this compound depletes dopamine from the striatum (Ferrano,1986; Grunblatt,1999; Miller, 1999). In our experiments, MPTP injection produced a severe oxidative stress in brain tissue. Firstly, MPTP caused significant increase in lipid peroxidation products (malondialdehyde + 4-hydroxyalkenals, MDA + 4-HDA) in striatum (Figure 3.) and hippocampus (Figure 4.). Pre-injection of UPF1 (as well as antioxidant melatonin) abolished the MPTP-dependent increases in lipid peroxidation products in striatum and hippocampus, whereas UPF1 effects are expressed more intensively (Figure 3. and Figure 4.).

Secondly, after MPTP administration also other oxidative stress indices as glutathione redox ratio (GSSG/2GSH) and total antioxidative activity were also crucially changed in striatum (Figure 5. and Figure 6.). The GSSG/2GSH was 2 times higher and total antioxidative activity about 3 times lower in striatum compared with control. Thus, both the levels of GSH and antioxidative total potency of striatum were crucially reduced after MPTP administration. Pre-treatment with UPF1, as well as with melatonin, prevented MPTP-induced effects and practically restored a normal level of GSSG/2GSH and total antioxidative activity (TAA) in striatum.

Thirdly, glutathione redox status was significantly increased in frontal cortex after injection of MPTP (Figure 7.). Pre-treatment with UPF1 prevented MPTP-induced influence and restored a normal level of GSSG/2GSH. Thus, UPF1, a new analogue of GSH, exhibited a crucial antioxidative protective action against neurotoxicity in MPTP model of Parkinson's disease model.

Thus, UPF1, a new analogue of GSH, exhibited a crucial antioxidative protective action against neurotoxicity in MPTP model of Parkinson's disease model.

MPTP is converted to MPP+ in the glial cells by monoamine oxidase and MPP+ accumulates selectively in dopaminergic neurons where its toxic action realizes via incompletely understood mechanisms. However, a possible mechanism involves both an induction of generation of several reactive species (including lipid hydroperoxides and alkenals) and depletion of endogenous defenses like a GSH (Poirier, 1985; Ferrano, 1986; Rojas,1993; Acuna-Castroviejo,1997). Thus, any designed new compound which is able to prevent the above mentioned MPTP-induced abnormalities might be have an impact as a possible neuroprotectant.

In summary, it has been shown that a new glutathione analogue of the invention (called UPF1) exhibits crucially higher hydrophobicity and antioxidativity compared with natural GSH. This compound has a potent free radical scavenging effect. UPF1 abolished the effect of toxic Aβ(25-35) on adenylate cyclase activity in rat frontal cortex membranes in Alzheimer'disease

model and revealed a significant suppressing influence in case of MPTP Parkinson's disease model *in vivo*.

The cited literature is incorporated herein by reference.

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Claims

1. Glutathione analogue having the formula

X-Glu -Y-Gly -Z

wherein Glu is glutamyl or γ-glutamyl, and Gly is glycine or glycyl,

X is selected from the group consisting of H-; (O-R)tyrosyl; biotinyl-(O-R)tyrosyl; anthraniloyl-(O-R)tyrosyl; (O-R) tyrosyl-aminohexanoyl-; and 5-alkoxy tryptophyl-;

Y is selected from the group consisting of cysteinyl, serinyl, methionyl or selenocysteinyl; and

Z is selected from the group consisting of -H; (O-R)tyrosine; and aminohexanoyl-(O-R)tyrosine,

and wherein each amino acids can be either a L- or a D- enantiomer; the peptide bonds may optionally be methylated, and the carboxyl groups are free acids, amides, or mono- or di-alkyl esters, and R is selected from alkyl, alkenyl, aryl and aryl containing one or several heteroatoms in the ring(s), with the provisio that X is not H when Y is L-cysteinyl and Gly is L-glycyl.

2. Glutathione analogue according to claim 1, wherein each alkyl group and the alkyl of the alkoxy group is selected from alkyl groups with from 1 to 20 carbon atoms; each alkenyl group is selected from alkenyl groups with 2 to 20 carbon atoms; each aryl group is selected from phenyl, benzyl, tolyl, xylenyl,naphtyl, anthranoyl or their substituted analogues, and each aryl group containing one or several heteroatoms in the ring(s) is selected from pyrrol, imidazole, thiazole, pyridine, pyrimidine, quinoline, indole, purine and their substituted analogues.

3. Glutathione analogue according to claim 2, wherein each alkyl group is methyl and the alkoxy group is methoxy.

4. Glutathione analogue according to claim 1, which is selected from the group consisting of Tyr(Me)-γ-Glu-Cys-Gly,

γ-Glu-Cys-Gly-Tyr(Me),

D-Tyr(Me)-γ-Glu-Cys-Gly amide, and

γ-Glu-Cys-Gly-Tyr(Me) amide.

5. Glutathione analogue according to claim 1, which is selected from biotinyl and anthraniloyl N-terminally substituted Tyr(Me)-γ-Glu-Cys-Gly ; γ-Glu-Cys-Gly-Tyr(Me); D-Tyr(Me)-γ-Glu-Cys-Gly amide; and γ-Glu-Cys-Gly-Tyr(Me) amide.

6. Glutathione analogue according to any one of claims 1 – 5 for use as an antioxidant.

7. Use of a glutathione analogue according to any one of claims 1-5 for the manufacture of a medicament for the prophylactic and/or therapeutic treatment of a disease or disorder associated with oxidative degeneration of cells.

8. Use of a glutathione analogue according to claim 7, wherein the disease or disorder is selected from Alzheimer's disease, Parkinson's disease, sunburn, adult respiratory distress syndrome (ARDS), cystic fibrosis, idiopathic pulmonary fibrosis (IPF), asthma, hyperoxia, HIV infection, influenza, Sjögren's syndrome, heart disease, epilepsy (Unverricht-Lundborg type), chronic bronchitis, and cancer.

9. A method of treating a disease or disorder associated with oxidative degeneration of cells in an animal or human comprising administration of a prophylactically or therapeutically effective amount of a glutathione analogue according to any one of claims 1-5 to said animal or human.

10. The method according to claim 9, wherein the disease or disorder is selected from Alzheimer's disease, Parkinson's disease, sunburn, adult respiratory distress syndrome (ARDS), cystic fibrosis, idiopathic pulmonary fibrosis (IPF), asthma, hyperoxia, HIV infection, influenza, Sjögren's syndrome, heart disease, epilepsy (Unverricht-Lundborg type), chronic bronchitis, and cancer.

11. A pharmaceutical preparation comprising a glutathione analogue according to any one of claims 1-5, and a pharmaceutically acceptable carrier.

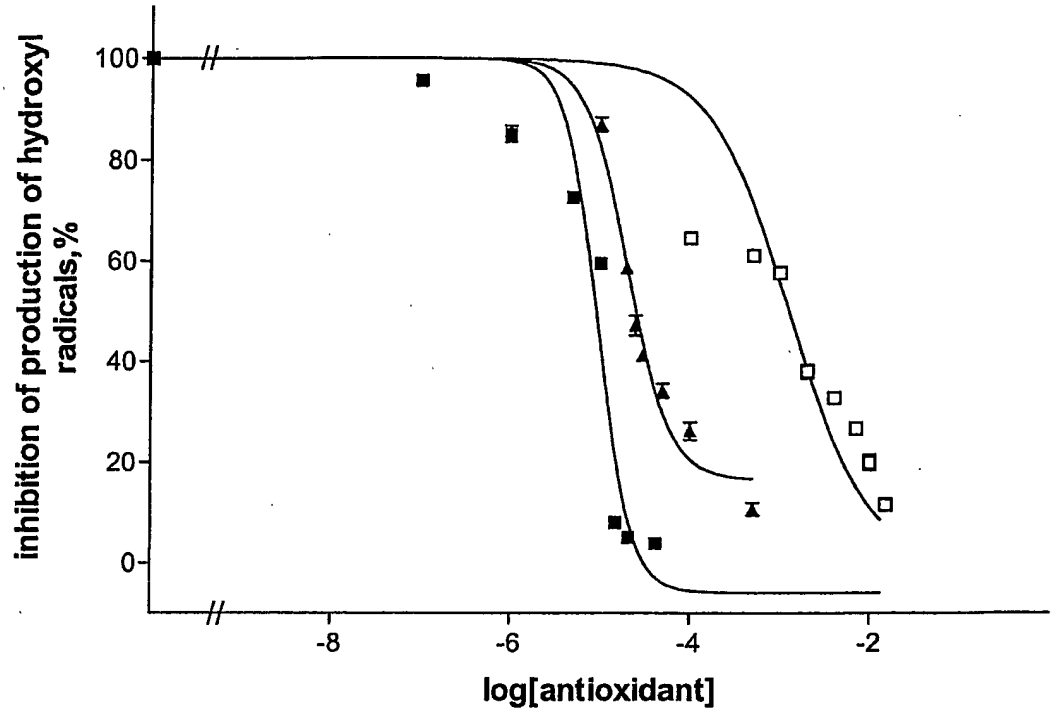
inhibition of production of hydroxyl radicals,%
100
80
60
40
20
0
-8
-6
-4
-2
log[antioxidant]

Fig. 1

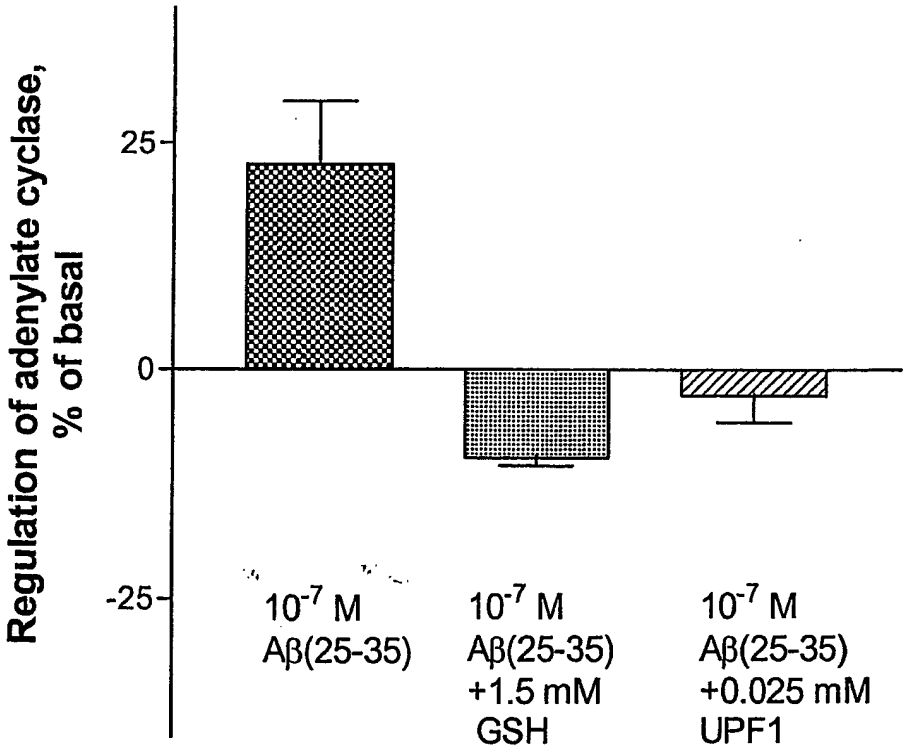
Regulation of adenylate cyclase, % of basal
25
0
-25
10-7 M Aβ(25-35)
10-7 M Aβ(25-35) +1.5 mM GSH
10-7 M Aβ(25-35) +0.025 mM UPF1

Fig. 2

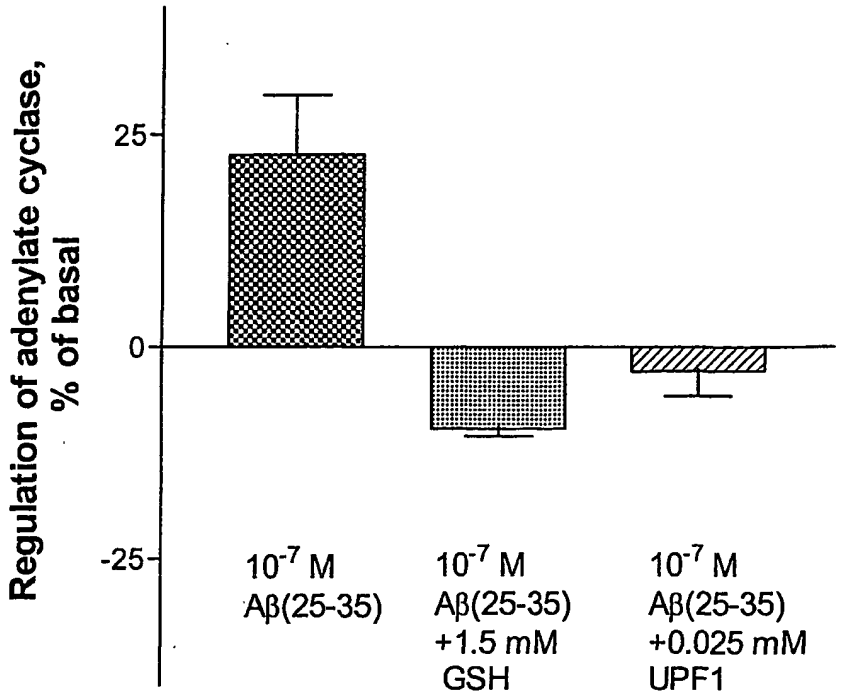
Regulation of adenylate cyclase, % of basal
25
0
-25
10-7 M Aβ(25-35)
10-7 M Aβ(25-35) +1.5 mM GSH
10-7 M Aβ(25-35) +0.025 mM UPF1

Fig. 3

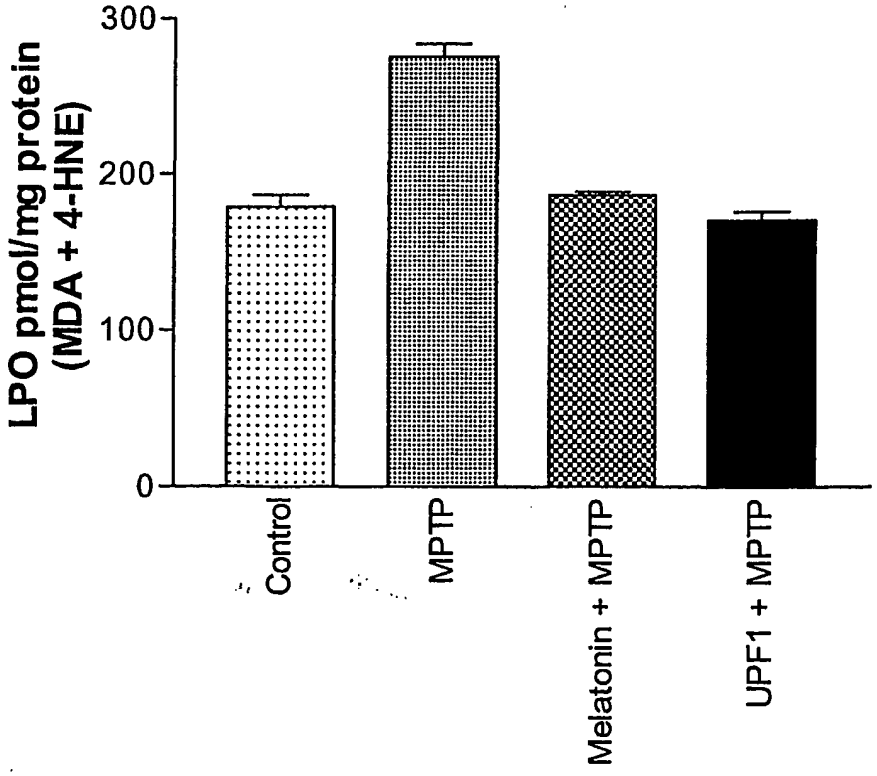
LPO pmol/mg protein (MDA + 4-HNE)
300
200
100
0
Control
MPTP
Melatonin + MPTP
UPF1 + MPTP

Fig. 4

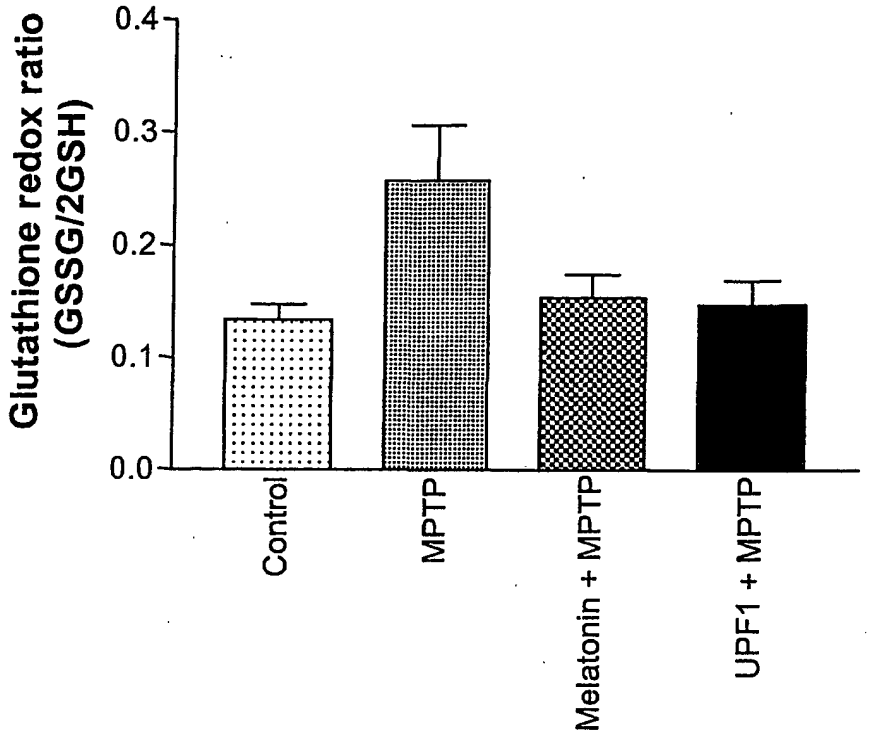
Glutathione redox ratio (GSSG/2GSH)
0.4
0.3
0.2
0.1
0.0
Control
MPTP
Melatonin + MPTP
UPF1 + MPTP

Fig. 5

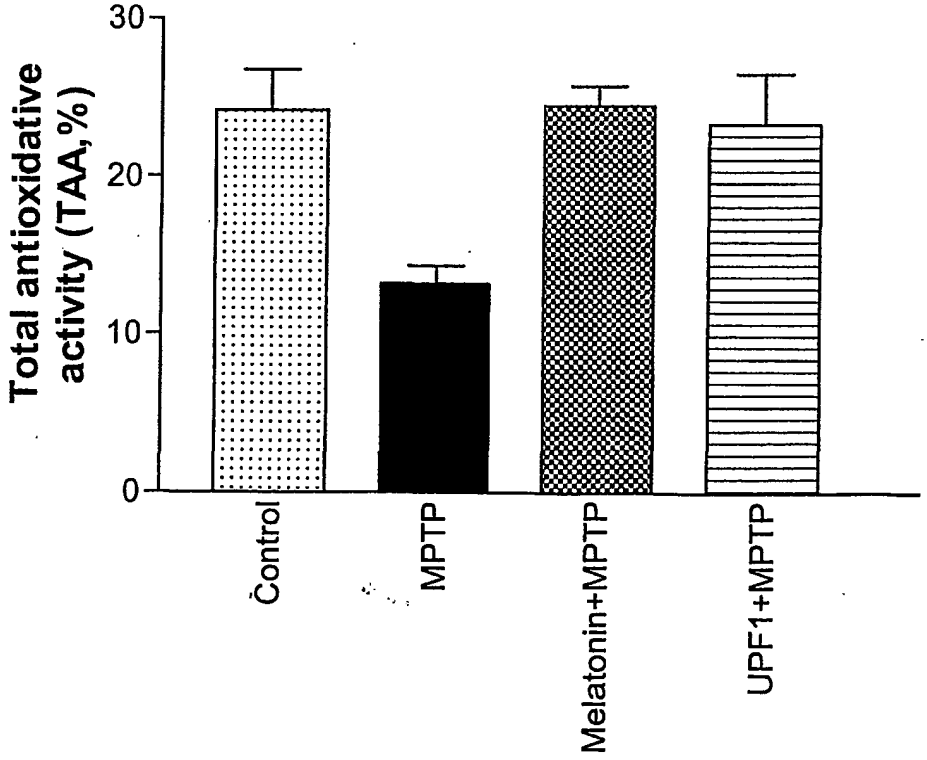
Total antioxidative activity (TAA,%)
30
20
10
0
Control
MPTP
Melatonin+MPTP
UPF1+MPTP

Fig. 6

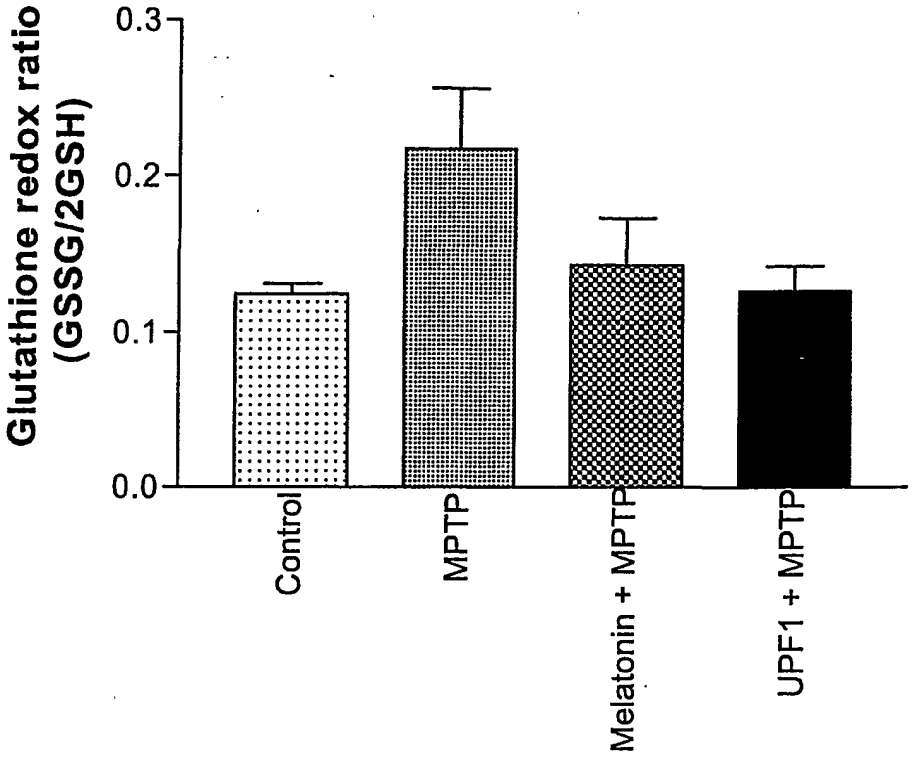
Glutathione redox ratio
(GSSG/2GSH)
0.3
0.2
0.1
0.0
Control
MPTP
Melatonin + MPTP
UPF1 + MPTP

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 01/01351

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 7/06, C07K 5/10, A61K 38/08, A61P 25/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI DATA, EPO INTERNAL, CHEM.ABS.DATA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5599903 A (KAUVAR ET AL), 4 February 1997 (04.02.97) --	1-11
A	US 4618669 A (DEREU ET AL), 21 October 1986 (21.10.86) --	1-11

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 November 2001	13 -11- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf/BS Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/10/01

International application No.
PCT/SE 01/01351

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5599903 A	04/02/97	AT 193302 T	15/06/00
		AU 693807 B	09/07/98
		AU 7842194 A	10/04/95
		CA 2171453 A	30/03/95
		DE 69424678 D,T	07/09/00
		DK 720620 T	07/08/00
		EP 0720620 A,B	10/07/96
		SE 0720620 T3	
		ES 2148348 T	16/10/00
		GR 3033230 T	31/08/00
		JP 9506336 T	24/06/97
		PT 720620 T	30/11/00
		US 5556942 A	17/09/96
		US 5763570 A	09/06/98
		US 5767086 A	16/06/98
		US 5786336 A	28/07/98
		US 5955432 A	21/09/99
		US 6013462 A	11/01/00
		WO 9508563 A	30/03/95
		US 5545621 A	13/08/96
		US 5679643 A	21/10/97
US 4618669 A	21/10/86	AT 41418 T	15/04/89
		DE 3422962 A	02/01/86
		DE 3568775 D	00/00/00
		DK 156053 B,C	19/06/89
		DK 282985 A	23/12/85
		EP 0165534 A,B	27/12/85
		SE 0165534 T3	
		GR 851465 A	25/11/85
		IE 58646 B	03/11/93
		JP 61050963 A	13/03/86
		ZA 8504708 A	26/02/86
		DE 3443468 A	28/05/86

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE01/01351

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 9-10
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Claims 9-10 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.